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Temperature requirements of *Alnus* pollen in Spain and Italy (1994–1998)

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Woody plants in temperate regions, in order to prevent the water in their cells from freezing, interrupt their growth entering into a physiological state called “dormancy”. Trees also have a heat requirement that must be fulfilled before actual growth is resumed and pollination occurs.

The objective of this study is to ascertain the influence that a climatic parameter such as temperature exerts on *Alnus*, in order to identify the start of the dormancy period, its duration and the consequent heat requirement that triggers flowering in three European regions located at the same latitude. In this regard, we chose two areas in Spain (Vigo and Santiago de Compostela) and one in Italy (Perugia), since they have different temperature patterns, ranging from mild in the case of Vigo to cold in that of Perugia, including an intermediate temperature pattern (Santiago).

Monitoring in the three stations was carried out by means of a 7-day Lanzoni VPPS 2000 pollen trap. *Alnus* began its pollen season between the second and third week of January in Spain, while in Italy it began during the first week of February.

During the four years under study, in order to overcome the dormancy period, Perugia needed an average of 962 chilling hours (CH), Santiago 622 CH, and Vigo had the smallest chilling accumulation with only 460 CH. In the case of heat accumulation, we found that Perugia had the smallest requirement and Vigo the largest, thus Perugia needed an average of 42 (growth degree days) GDD, Santiago around 50 GDD and Vigo 62 GDD.

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A study of the physiological behaviour of plants in non-equatorial areas shows that the seasonal climatic parameters that influence most of these processes are subject to constant variations, being more noticeable in temperate regions. Such unstable climatic conditions mainly involve light intensity, photoperiod, temperature and precipitation. Consequently, there are favourable and non-favourable periods, generally seasonal phases, which exert different kinds of influence on the life cycles of the so-called superior plants.

Plant requirements, in order to survive periods of adverse climatic conditions, are satisfied by means of physiological adaptations. Thus, woody plants in temperate regions, in order to prevent the water in their cells from freezing, slow their growth down almost to a standstill, entering into a physiological state called “dormancy”. Dormancy may be defined as the state in which growth is temporally interrupted (Wareing & Phillips 1978). The term “forced dormancy” can be used when it is brought on by unfavourable climatic conditions. Of all the aforementioned meteorological parameters, the majority of winter-flowering tree species in temperate regions are mainly influenced by temperature and photoperiod, the former before flowering and the latter during it (Kramer & Kozłowski 1979).

Many authors indicate that the end of the dormancy phase and the start of the growth of male inflorescences in the

majority of woody plants, require a period of low temperatures followed by another of relatively warm temperatures (Wareing & Phillips 1978, Felker et al. 1983, Faust 1989). It is very difficult to predict exactly the moment when the adverse period will end and the flower will be prepared for anthesis. The study of specific morphological and cytological parameters, such as the growth pattern of the catkin or male microspore before pollination, can be used to determine the start and end of dormancy, which defines the vegetative state. An increase in size can provide us with the key to a better understanding of the process.

At an ultrastructural level, there are no noticeable changes in dormant leaf buds compared to others (only the endoplasmic reticle and nucleus appear to be involved, and the shape and general disorganization of the latter suggest that the entire process may be due to water stress), but an important morphological change during the dormancy period could be due to the variation of the pollen grain size, which can be used as an indicator defining this vegetative phase (Felker et al. 1983, Ferranti et al. 1996, Frenguelli et al. 1997, Frenguelli & Brichi 1998). In winter-flowering woody plants, the diameter decrease of the microspore begins two weeks before the onset of dormancy (Frenguelli 1997), thereafter remaining constant during the period of low temperatures. The pollen volume increases again just before

anthesis, reaching its maximum value before decreasing slightly at the start of anthesis (Ferranti et al. 1996). The period of full dormancy is then identified when the microspore volume decreases by about 12% as a result of dehydration. An important role is also played by the oncus, very big in *Alnus* pollen grains, which, since they are water reservoirs, are capable of increasing and adapting to size variations (Pacini & Franchi 1984). The plasmatic membrane is capable of regulating water flow (Basani et al. 1994). This is a defence mechanism against low temperatures, which cause freezing within the reproductive structures. When the adverse period is over, the pollen volume increases again by means of rehydration to the mature state that enables anthesis. We can therefore differentiate this moment by observing the oncus structure (Ferranti et al. 1996). These hydration-rehydration variations may be very useful in ascertaining the moment a plant begins and ends dormancy.

The objective of this study is to ascertain the influence that a climatic parameter such as temperature exerts on *Alnus*, in order to identify the start of the dormancy period, its duration and the consequent heat requirement that triggers flowering in three European regions located at the same latitude. It is extremely important to accurately ascertain the chilling and heat requirements that satisfy plants of the *Alnus* genus, so that anthesis may take place in the different climatic regions, in order to predict the start of pollination in these winter-flowering plants. In this regard, we chose two areas in Spain (Vigo and Santiago de Compostela) and one in Italy (Perugia), since they have different temperature patterns, ranging from mild in the case of Vigo to cold in that of Perugia, including an intermediate temperature pattern (Santiago de Compostela).

MATERIALS AND METHODS

The start of the *Alnus* pollination period was studied by monitoring atmospheric pollen concentrations in three European regions (Fig. 1), from 1995 to 1998 inclusive.

The monitoring station in the centre of Italy was Perugia (with a sub-Mediterranean climate), at an altitude of 493 m and located approximately 90 km from the Mediterranean Sea on the west-facing slopes of the Apennines. It is surrounded on the southwest by the valley of the River Tiber and on the northwest by Lake Trasimeno. It has a mean annual temperature of 13.2°C, a mean maximum temperature of 17.1°C and a mean minimum temperature of 9.2°C. It has 877 mm of annual precipitation.

Santiago de Compostela, northwest Spain, is located at 270 m above sea level in the valley of the River Ulla 50 km from the Atlantic Ocean, with a temperate maritime climate. It has a mean annual temperature of 12.9°C, with mean maximum and minimum temperatures of 17.1°C and 8.8°C respectively. It has a total annual precipitation of 1,288 mm. As regards the city of Vigo, it is situated at sea level on the right-hand shore of the Vigo estuary. The valley making up the city has a small drainage system, mainly running N-S and SW-NE. Like Santiago, it has a temperate maritime climate, but in this case the mean annual temperature is 14.9°C, the mean maximum temperature is 18.8°C and the mean minimum temperature is 11°C. It has the highest total annual precipitation with 1,412 mm.

With regard to sources of *Alnus* pollen, in Italy these are mainly trees of the *Alnus glutinosa* and *Alnus incana* species, while in Spain only the *Alnus glutinosa* species is found, making up the dominant formations alongside river beds, accompanied by *Betula celtiberica* and *Salix atrocinerea*. Frequently, alder trees are a common ornamental vegetation in the gardens and streets of these cities.

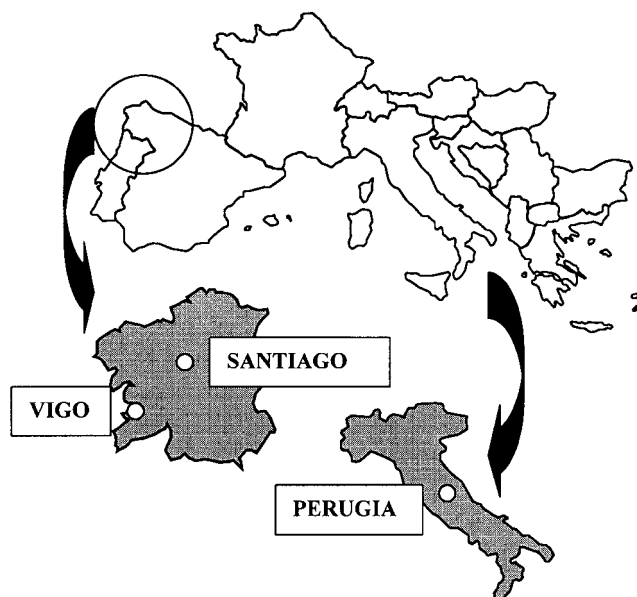


Fig. 1. Location of the zones of study.

Monitoring in the three stations was carried out by means of a 7-day Lanzoni VPPS 2000 pollen trap, based on the Hirst pollen trap (Hirst 1952). In Perugia it was located on the roof of the Faculty of Agronomic Science, in Santiago on the roof of the Faculty of Pharmacy and in Vigo on the terrace of the town hall. Melinex tape soaked in a 2% silicone solution was used as the trapping surface. The mean daily values were represented as the number of pollen grains per cubic metre of air.

The meteorological data used for the study were recorded in Perugia by the Faculty of Agronomic Science's Institute of Ecology, and in Santiago and Vigo by the National Institute of Meteorology.

In order to obtain the pollination period we took into account the periods covering 98% and 95% of total annual pollen, eliminating the initial period until the 5-day running mean exceeded 1% (Mullenders 1974) and 2.5% (Andersen 1991).

We used the model proposed by Aron in 1983 for the calculation of the chilling requirement, based on the accumulation of chilling hours between 0 and 7.2°C. In this study we began to calculate the accumulation of chilling hours (CH) in November, since it is the time of the year when the mean temperature drops below 12.5°C, the temperature below which winter-flowering woody plants are considered to begin to satisfy their chilling requirements. In the case of the heat requirement for pollination, it is expressed as GDD (growth degree days) in °C, obtained from the sum of the daily mean temperatures after deducting different base temperatures of 4.5°, 5°, 5.5° and 6°C, which represent the thresholds above which the temperature begins to be effective for growth (Faust 1989).

RESULTS

After calculating the pollination periods, in order to carry out the study we chose the period described by Andersen (1991), which includes 95% of total annual pollen, eliminating the initial period until the 5-day running mean did not exceed 2.5% of the total *Alnus* pollen recorded. We chose this method, since with it there was a smaller standard deviation in relation to the start dates of *Alnus* flowering during the years under study than with Mullenders' method (Table I).

Alnus began its pollination period between the second and

Table I. Numbers of the starting day (from the first of January) of the *Alnus* pollen season calculated with 2.5% (Andersen 1991) and 1% (Mullenders 1974) methods.

Season	Perugia		Santiago		Vigo	
	1%	2.50%	1%	2.50%	1%	2.50%
1994-95	30	37	9	13	11	12
1995-96	26	31	20	20	4	14
1996-97	33	35	13	14	11	13
1997-98	32	36	10	10	9	10
Mean	30	35	13	14	9	12
Minimum	26	31	9	10	4	10
Maximum	33	37	20	20	11	14
Standard deviation	3.10	2.63	4.97	4.19	3.30	1.71
Coefficient std. var. %	10.23	7.57	38.20	29.43	37.76	13.94

third week of January in Spain, while in Italy it began during the first week of February (Fig. 2). In Vigo flowering took place the earliest and a few days later in Santiago, while in Italy there was an average delay of around two weeks compared to the dates recorded in Spain. All of the stations

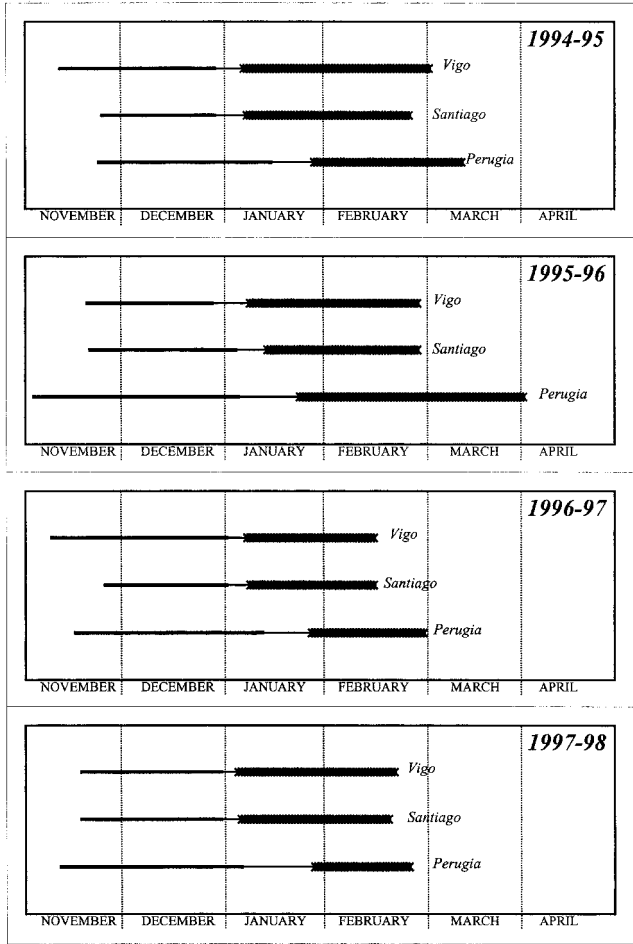


Fig. 2. Chilling hours (—), Growth degree days (—) and Polination Period (■) for Perugia, Santiago and Vigo during period under study.

followed the same pattern during the years under study, with less variation in the start date of flowering between the westernmost stations.

In order to obtain chilling accumulation expressed as chilling hours (CH), which provides us with a relationship between recorded temperatures and the effective temperature for the developing catkin, we took into account the moment when the mean temperature dropped below 12.5°C, the temperature considered by Richardson et al. (1974) as the start of the chilling hours count. The values were different in the three cities, probably due to the cold climatic parameters in Perugia and the temperate ones in Santiago and Vigo (Fig. 3). Thus, Vigo had the smallest requirement and Perugia the largest, with Santiago in the middle (Table II). During the four years under study, in order to overcome the dormancy period, Perugia needed an average of 962 CH, Santiago 622 CH and Vigo had the smallest chilling accumulation with only 460 CH. We found a statistically smaller CH standard deviation coefficient in the case of Perugia and Vigo.

The different heat accumulations expressed as GDD°C, all of which were calculated following Faust's method (Faust 1989) but with different base temperatures of 4.5°, 5°, 5.5°

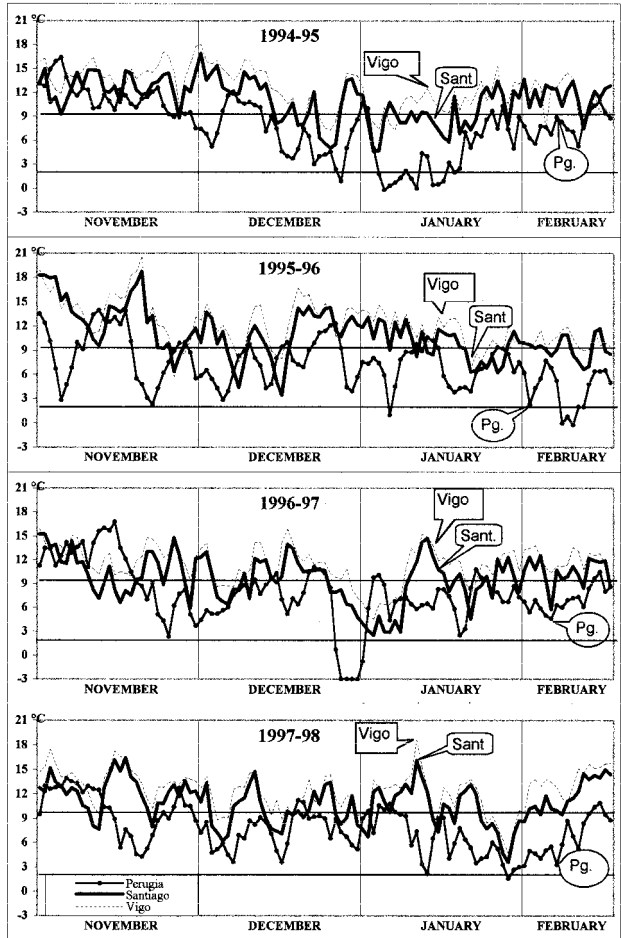


Fig. 3. Mean temperature for Perugia, Santiago and Vigo from November to February during period under study.

Table II. *Alnus* CH during period under study.

Chilling hours	Perugia	Santiago	Vigo
1994–95	940	568	463
1995–96	962	542	440
1996–97	981	825	487
1997–98	923	551	451
Mean	952	622	460
Minimum	923	542	440
Maximum	981	825	487
Standard deviation	25.33	136.09	20.16
Coefficient std.var.%	2.66	21.90	4.38

and 6°C (Table III). The values also varied considerably in the three cities. There was a smaller standard deviation coefficient in the heat requirement calculations with a base temperature of 4.5°C. In the case of heat accumulation, we also found constant behaviour during the years under study, since the colder the temperatures in the region, the less heat requirements there are; Perugia had the smallest requirement and Vigo the largest, thus Perugia needed an average of 42 GDD (4.5°C base temperature) during the three years under study, Santiago a constant heat accumulation of around 50 GDD (with the smallest standard deviation) and, finally, Vigo needed the highest average of degree days to attain the necessary heat in order to start flowering with 62 GDD.

Finally, in Fig. 4, we can see the correlation between the accumulation of chilling hours during dormancy and the heat required to produce pollination, giving an *r* value of 0.497 with *p*<0.1. When the quantity of chill is calculated as the accumulation of chilling hours during autumn, it is clear that the plants of the colder stations accumulate a greater quantity of chilling to produce pollination. For its part, the heat accumulation was similar in all three cities.

There is an extremely high correlation between the start dates of the *Alnus* pollen season and CH accumulation, with an *r* value of 0.880 and *p*<0.01 (Fig. 5).

DISCUSSION

The temporal situation of pollination is due to important and successive requirements of temperature (Frenguelli et al.

	GDD–CH
r	0,4970
r ²	0,2470
p	0,0999

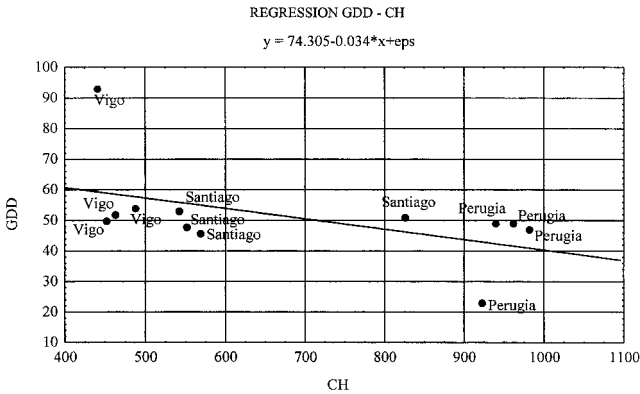


Fig. 4. Relationship between chilling hours accumulated during dormancy and heat (GDD°C base 4.5) required for *Alnus* pollination.

	CH-START	GDD-START
r	0,8800	0,395
r ²	0,7740	0,156
p	0,0002	0,232

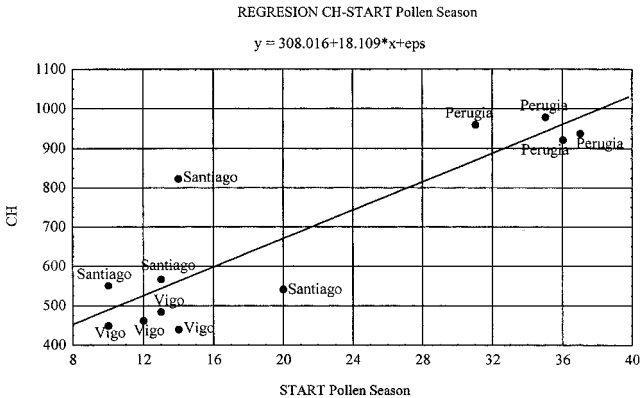


Fig. 5. Relationship between chilling hours accumulated during dormancy and start of *Alnus* pollination.

Table III. *Alnus* GDD for Perugia, Santiago and Vigo during period under study using different base temperatures.

	Perugia				Santiago				Vigo			
Growth Degree Days (in calc. °C)	4.5	5	5.5	6	4.5	5	5.5	6	4.5	5	5.5	6
1994–95	49	42	34	27	46	41	36	31	52	48	43	39
1995–96	49	42	35	28	53	48	43	38	93	87	81	75
1996–97	47	39	32	26	51	48	44	41	54	51	48	45
1997–98	23	17	12	9	48	45	42	39	50	48	45	43
Mean	42	35	28	23	50	46	41	37	62	59	54	51
Minimum	23	17	12	9	46	41	36	31	50	48	43	39
Maximum	49	42	35	28	53	48	44	41	93	87	81	75
Standard deviation	12.70	12.08	10.90	9.04	3.11	3.32	3.59	4.35	20.56	19.05	17.95	16.52
Coefficient std. var.%	30.24	34.52	38.60	40.16	6.28	7.29	8.71	11.68	33.04	32.57	33.09	32.72

1991, Spieksma et al. 1989). In preceding studies carried out in these areas of study, a statistically significant correlation was found between the start of the pollination period and the addition of the mean temperatures registered during the 20, 30 and 40 days before the flowering ($p < 0.01$ in most of the cases). These data show a sufficiently strong significance to get to the conclusion that temperature exerts a great influence on the start of flowering. To get more information, we have studied the heat and chilling requirements that *Alnus* needs to break the dormancy period that undergoes during the previous months to the start of the flowering.

The delays in the start date can be explained both by the climatic differences of the areas under study and by the fact that these requirements can be genetically determined, and that in each area the trees belong to different genetic populations. Thus, throughout the years of study, it has been a constant that the places with colder temperatures present a higher chilling requirement and a higher heat accumulation, since in these areas, the trees need to protect themselves for longer periods. We can check how this pattern of behaviour is fulfilled from a colder place such as Perugia, to a warmer one such as Vigo, with an intermediate one such as Santiago.

At a particular level, and inside each area, the differences in the start of the flowering throughout the years can be explained both by a high temperature during the chilling period, and by a lower temperature during the heat accumulation. This effect is obvious when observing the CH obtained in Santiago. While in Perugia and Vigo the start dates of flowering have a low coefficient of standard variation, in the case of Santiago this is much higher, reaching almost 22%. The reason is the warm autumn in this city in 1996 that produced a great need of chilling accumulation.

Regarding the heat accumulation, the high mean value of 62-degree days that were needed in Vigo to reach the necessary heat for the start of the flowering is strongly influenced by the temperature events in 1996. Due to the low temperatures registered at the heat accumulation moments during this year, more days than in other years were needed, finally reaching a larger quantity of GDD.

We should also emphasise the fast heat accumulations registered in Vigo and Santiago during the last two years of study. These motivated an advance in the flowering, especially in 1998.

Finally, the results obtained from the regression line between the accumulation of CH and the GDD represented in Fig. 3 reinforce the assertion that the places with colder temperatures present a higher chilling requirement and a higher heat accumulation. At an extreme of the regression line we have the points that correspond to the values reached during the years of study in Vigo, and at the other extreme those of Perugia, being in the central part those corresponding to Santiago.

A datum that emphasises the importance of the chilling accumulation in this process is the high correlation between the start dates of the pollinic season of the *Alnus* and the accumulation of growing degree-days. This seems to indicate that for the pollination of this winter-flowering plant, the chilling requirements are more important and they exert a more important influence than heat.

CONCLUSIONS

Alnus pollen season has started between the second and third week of January in the Spanish cities, while in the Italian city of Perugia, it began during the first week of February.

During the four years of study it has been proved that in order to overcome the dormancy period, Perugia needed an average of 962 chilling hours, Santiago 622 CH. Vigo had the smallest chilling accumulation with only 460 CH. In the case of heat accumulation, it has been proved that Perugia had the smallest requirement and Vigo the largest: Perugia needed an average of 42 GDD, Santiago around 50 GDD, and Vigo 62 GDD.

As a conclusion, we may say that as it has been stated before (Frenguelli et al. 1991, Spieksma et al. 1989), there are two main influences of temperature on the pollination date of the woody plants of the temperate area. The first one is that the trees require a chilling period before they reach the end of the dormancy, letting the breaking of the catkins, and the second one is that the trees also have a heat requirement that can be satisfied before the current growth restarts and the pollination takes place.

These data show that the differences that appear in relation to the start of the pollen season for different species of trees in the temperate area in different climate regions, can be explained by the temperature of the air during the periods preceding the pollen season. A parallel phenological research would be necessary to obtain a greater knowledge of this phenomenon.

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